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# EVALUATION OF PROTEIN BAIT FORMULATIONS FOR THE CARIBBEAN FRUIT FLY (DIPTERA: TEPHRITIDAE)

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#### ABSTRACT

Laboratory and field trials were conducted to determine the preference of the Caribbean fruit fly, Anastrepha suspensa (Loew), for aqueous formulations of the protein bait NuLure® and standard torula yeast plus sodium borate (HTY-borax) pellets. Addition of 1-10% borax to 10% NuLure solution increased bait pH, and this increase was directly correlated with increase in number of female flies trapped in two-choice laboratory bioassays and in field trials conducted in three locations in south Florida during the spring of 1992. Overall, significantly more flies were attracted to volatiles from HTY-borax solution than to volatiles from any of the NuLure solutions. There was variation in the response of flies to baits observed among the three test locations. Age structures of the populations at the different locations were compared by determining

the percentage of females mated among the trapped females. There were significant differences in the percentage of mated females at the 3 locations, which ranged from 26-65% mated. In the locations with a high percentage of unmated female flies, the flies were less discriminating, at least among the protein baits offered in this study. Thus, age structure of the target population may alter response to bait. There was also variation in the pH of the baits at different locations due to the pH of the water used to prepare the solutions. Bait pH may be a significant factor that has been overlooked in field tests conducted at different locations or at different times as changes in water pH, as well as bait protein pH, may strongly affect trap efficacy.

Key Words: Anastrepha suspensa, Tephritidae, protein baits, trapping, bait pH.

#### RESUMEN

Se llevaron a cabo ensayos de laboratório y de campo para determinar la preferencia de la mosca caribeña de las frutas, Anastrepha suspensa (Loew), por formulaciones acueas del cebo proteinaceo NuLure® y levadura torula estandard mas pellotillas de borato de sodio (TY-borax). La adición de 1-10% borax a una sulución de 10% de NuLure aumento el pH del cebo, y este aumento fué corelacionado directamente con el aumento en el número de hembras de las moscas entrampadas en bioensayos de laboratorio de dos surtidos y en ensayos de campo llevado a cabo en 4 localidades in el sur de Florida durante la primavera de 1992. Por lo general, significativamente mas moscas fueron atraidas a los volatiles de la solucion TY-borax que a los volatiles de las soluciones de NuLure. Hubo una variación en la respuesta de las moscas a los cebos observados entre las tres localidades de las pruebas. Las estructuras de las edades de las poblaciones en las diferentes localidades fueron comparadas para determinear el porcentage de las hembras pareadas entre las hembras entrampadas. Hubieron diferencias significativas en el porcentage de las hembras pareadas en las tres localidades, lo cual varió entre 26 hasta 65% pareadas. En las localidades con un alto porcentage de moscas hembras no pareadas, las moscas fueron menos discriminatorias, por lo menos entre los cebos proteináceos ofrecidos en este estudio. Así, la estructura de las edades de la población objectiva puede alterar la respuesta al cebo. Hubo tambien variación en el pH de los cebos en diferentes localidades, debido al pH del agua usada para preparar las soluciones. El pH del cebo puede ser un factor significativo, de lo cual no se ha hecho caso en pruebas de campo llevado a cabo en diferentes localidades o durante diferentes periodos, porque cambios en el pH del agua, igual como pH del cebo proteináceo, puede afectar fuertamente la eficiencia de la trampa.

The presence of the Caribbean fruit fly (caribfly), Anastrepha suspensa (Loew) (Diptera: Tephritidae), in citrus growing regions of Florida is of considerable economic importance. Even a small infestation of flies in an area creates a quarantine problem and renders citrus unsalable to many potential domestic and foreign markets without costly post-harvest treatments (Greany & Riherd 1993, Simpson 1993). Because of this threat and the potential for introduction of the caribfly into current fly-free areas, much emphasis has been placed on detection of this species. The development of improved lures for monitoring and suppressing populations of the caribfly would safeguard current fly-free zones and permit expansion of these zones to other citrus growing regions in Florida.

Traps baited with proteins have been used historically to detect and monitor populations of *Anastrepha* fruit flies. Hydrolyzed proteins, often by-products of commercial operations, have been found to be superior to non-hydrolyzed proteins, and a number of hydolyzed and non-hydrolyzed proteins have been tested for attractant activity (Steiner 1955). The proteins in these food-based lures often provide nutrients critical

for female reproductive capability (Steiner 1955). Among the promising baits in those tests were enzymatic yeast hydrolysates and acid corn hydrolysates [i.e., NuLure (Miller Chemical and Fertilizer Co., Hanover, PA.), also known as Staley's, PIB-2 or PIB-7]. Addition of sodium borate (borax) was found to reduce decomposition of both the protein bait and the trapped flies (Lopez-D & Becerril 1967), and pelleted formulations were developed to facilitate field placement (Lopez-D et al. 1968).

Bait preference varies among the Tephritidae. Anastrepha ludens (Loew) preferred cottenseed hydrolysate over PIB-7 (Lopez-D & Becerril 1967). A. striata (Shriner), A. serpentina (Wiedemann), A. obliqua (Macquart) and A. balloui (Stone) preferred traps baited with hydrolyzed soy protein plus borax over traps with torula yeast plus borax or borax alone (Jiron and Soto-Manitiu 1989). Lopez-D et al. (1971) compared several hydrolyzed torula yeast (HTY) and borax formulations and found that 3% HTY with 4% borax aqueous solutions attracted the greatest number of caribflies. Steiner (1955) found acid and enzymatic hydrolysates to be equal in attractiveness for the Mediterranean fruit fly, Ceratitis capitata (Wiedemann). Bateman and Morton (1981), however, found that addition of borax to acid hydrolysates increased bait pH and attraction of the Queensland fruit fly, Bactrocera tryoni (Froggatt). The relationship between pH of the acid hydrolysates and catch of fruit flies has been largely unexplored.

McPhail traps, bell-shaped glass traps with a water reservoir (Newell 1936), baited with 5 to 6 HTY-borax pellets are currently used for detection and delineation of the caribfly in Florida (Anonymous 1989). Low catch efficiency (Calkins et al. 1984), high cost and difficulties in handling the cumbersome liquid-baited McPhail traps in the field have led to searches for more efficient attractants and traps. Identification of chemical attractants is critically dependent upon the availability of a bioassay to monitor relevant biological responses (Heath et al. 1993a). A volatile attractant bioassay system was developed in our laboratory that allows both the testing of volatiles from various substrates for attractant activity and the collection of volatiles from biologically relevant substrates for subsequent chemical identification. This paper reports studies of several protein baits, and compares results from laboratory bioassays with colony-reared flies to field trials with natural populations. We examined the effect of addition of borax to NuLure on bait pH and bait attractiveness to caribflies, and determined choice among standard HTY-borax solution and formulations of NuLure-borax.

#### MATERIALS AND METHODS

# Laboratory Bioassays

Caribbean fruit flies used in laboratory studies were obtained as pupae from the Florida Department of Agriculture and Consumer Services, Division of Plant Industry (FDACS-DPI) in Gainesville, Florida. Flies were given water and adult food, a 3:1 mixture of refined cane sugar:protein hydrolysate, and were maintained in screen cages (30 cm³) in a laboratory with a photoperiod of 12:12 (L:D) at room temperature and ambient humidity. Females used in laboratory tests were obtained from mixed-sex cages and ranged in age from 3-16 d post-eclosion. Females were protein-starved for 24 h prior to testing to standardize the physiological condition of insects to be tested (Hsiao 1985).

All bioassays were conducted using a two-choice volatile attractant bioassay system that is a modified wind-tunnel bioassay (Heath et al. 1993b). This system quantifies the effectiveness of various attractants by releasing volatiles from test substrates upwind of a group of test insects at a precise and controllable rate. The bioassay test chamber is a  $122 \times 30.5 \times 30.5$  cm plexiglass tunnel with 2 horizontally-mounted traps suspended inside the tunnel. Traps are attached symmetrically 5.1 cm left and right of the center-

line on the top panel of the bioassay test chamber 5.1 cm from the front (upstream) edge. Traps are made from clear plastic 140 ml vials (8.57 cm long × 4.76 cm ID) with a removable plastic snap lid (BioQuip, Gardena, CA) that has a 1.59 cm diameter hole drilled in the center. Volatiles from the test substrate enter the bioassay test chamber and responding insects enter the trap through this hole. The lid is orange-colored to provide a visual cue (Greany et al. 1977, Sivinski 1990) and an insert with Tanglefoot® is placed in the trap to retain the responding flies. There were 20-25 females tested per bioassay. Volatiles from the test substrates are vented for 1 h prior to the addition of flies to allow volatile release to stabilize. Number of flies per trap was recorded after approximately 20 h.

In preliminary tests, it was found that volatiles from water alone or from aqueous solutions of borax were not attractive. Therefore, all studies were two-choice preference tests with females exposed to volatiles from 2 protein bait formulations. The first study examined the effect of addition of borax to NuLure solution. Aqueous solutions, 100 ml each, of 10% NuLure with 0, 1, 5, and 10% borax were compared. There were 12 replicates per bait with each treatment tested against every other treatment an equal number of times. Fresh bait solutions were made after every 3 replicates. In the second study, insects were given the choice of volatiles from standard bait [1 HTY-borax pellet (ERA Int., Freeport, NY) in 100 ml water] or from 100 ml of 10% NuLure with 5% borax in water. Because of potential decrease in attraction by 6 d due to HTY-borax decomposition (Davis et al. 1984), fresh bait solutions were made every 4 d. There were 40 replicates per bait. Bait pH was measured every day (study one) or at the start and end of each 4-d period (study two) using a microprocessor benchtop pH meter (Omega Engineering, Inc., Stamford, CT).

The effect of bait formulation on the percentage of flies trapped per bait was analyzed by analysis of variance (ANOVA) using Proc GLM followed by Tukey's mean separation test (P=0.05) or by t-test (SAS Institute 1985). A paired-comparison t-test was conducted on the difference in percent trapped per bait (higher minus lower percent borax in the first study; HTY-borax minus NuLure in the second study) using Proc UNI-VARIATE (SAS Institute 1985). This test determined the significance of choice within the same tunnel.

#### Field Trials

Field trials were conducted at 8 sites in 3 locations in south Florida during spring 1992. At this time of year, Surinam cherry, Eugenia uniflora L., is in fruit and is the major host for caribflies. There were no other major hosts in fruit at this time. Four bait treatments, consisting of 300-ml solutions of 1) HTY-borax (3 pellets), 2) 10% NuLure plus 1% borax, 3) 10% NuLure plus 5% borax, 4) 10% NuLure plus 10% borax; and a control treatment [water], were tested. A water control was used because preliminary studies had indicated that borax solutions were no more attractive than water. A site consisted of 5 McPhail traps, with all treatments and a control placed around the periphery of a large single tree or planting of Surinam cherry in fruit. Trap placement within the site followed standard protocols (Anonymous 1989). There were 2 sites in Ft. Denaude, FL; 3 sites at the Univ. of Florida Tropical Research and Education Center (TREC) in Homestead, FL; and 3 sites at the Fruit and Spice Park in Homestead, FL. Traps were checked after 2, 4 and 7 days. At the time of sampling, flies were removed, bait pH was measured with a portable pH meter (pHep<sup>+</sup>, Cole-Parmer Instrument Co.), bait was reused (on days 2 and 4) or replaced (day 7), and traps were moved sequentially to a new position within the site. Numbers of male and female caribflies were recorded, and the flies were placed in 75% ethanol.

The mated status of females from the different locations was determined by dissecting a subsample of trapped females from HTY-borax and NuLure samples, and checking for presence or absence of sperm in the spermathecae. The NuLure-trapped flies used for dissection were obtained predominantly from traps of NuLure with 5% borax unless less than 10 females were caught, in which case females from NuLure with 1 or 10% borax were substituted. All flies dissected were obtained from the 0-1 d bait age samples, that is, from collections made on day 2. Subsamples, consisting of 10 flies dissected per sample, were obtained from 2 baits from each of the 8 sites and from 2 different weeks, for a total of 32 samples.

The total number of collections per location ranged from 33 at the Fruit and Spice Park to 36 from TREC. The tests were conducted from late April until early June, 1992. The trapping data were analyzed with three-way ANOVA with two-way interactions (factors: location [3 levels], bait formulation [4 levels] and bait age [3 levels]) using Proc GLM (SAS Institute 1985) with Tukey's mean separation test (P=0.05) used for significant factors. Data were transformed ( $\log \times + 1$ ) to meet the assumption of homogeneity of variance prior to analysis. The percentage of mated females was tested with two-way ANOVA (factors: location [3 levels], bait formulation [2 levels]) with interaction.

## RESULTS AND DISCUSSION

## Laboratory Bioassays

The effect of borax concentration in the 10% NuLure solution on female response was significant (F=6.37; df = 3, 44; P=0.001). Borax increased the pH of NuLure, and this increase was directly correlated with increase in percentage of flies trapped (Fig. 1). No matter which 2 borax treatments were compared in a bioassay tunnel, significantly more flies were attracted to the trap emitting volatiles from the NuLure solution with the higher pH (mean difference = 25%, t=5.94, df = 23, P=0.0001).

Significantly more flies were trapped with HTY-borax than 10% NuLure-5% borax (t=2.2, df=38, P=0.03). An average ( $\pm$  standard error of the mean [SEM]) of 29% ( $\pm$  2.8%) of the flies responded to volatiles from HTY-borax while 20% ( $\pm$  2.5%) responded to volatiles from NuLure-borax. The pH of the HTY-borax was higher than that of the NuLure-borax (pH 9.0 and pH 8.2, respectively).

# Field Trials

Very few flies were caught in the control treatments (i.e., < 5), so data from control traps were deleted for subsequent analyses. More female caribflies than males were trapped during these studies, and the ratio of females:males ranged from 2:1 at Fruit and Spice Park and Ft. Denaude to 5:1 at TREC. A female bias in HTY-borax baited McPhail traps was noted in studies of A. suspensa (Lopez-D et al. 1971) and other Anastrepha spp. (Malo 1992). Location significantly affected the number of males trapped (F=7.09; df = 2, 388; P=0.0009), but not the number of females (F=2.45; df = 2, 388; P=0.08). Fewer males were trapped at TREC, as indicated by the high female:male ratio in the trap catch. Data on the mated status of trapped females were compared among the populations from the three locations. There were significant differences in the percentage of mated females at the three locations (F=8.62; df = 2, 26; P=0.001). TREC had a higher percentage of mated females trapped than did either Ft. Denaude or Fruit and Spice Park (65%, 36% and 26%, respectively). There was a significant interaction between location and bait treatment for females (F=2.43; df = 4, 388; P=0.026). Therefore, separate analyses were conducted on the effect of bait

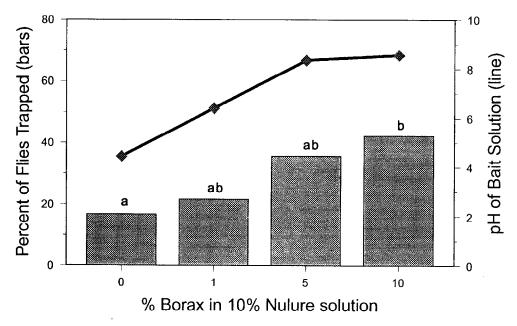


Fig. 1. Percent of female Anastrepha suspensa trapped (bars, n=12) in laboratory bioassays of response to volatiles from bait solutions of aqueous 10% NuLure solution with 4 concentrations of sodium borate (borax), and the differences in pH (line) of bait solutions. Bars headed by the same letter are not significantly different (Tukey's mean comparison, P=0.05).

treatment within each location. There were significant differences among the baits at TREC and Ft. Denaude (Fig. 2). The catch in the HTY-borax traps was significantly higher than all NuLure traps at TREC and the catch in the NuLure-1% borax traps was significantly lower than all other baits at Ft. Denaude.

Bait tested accounted for most of the variation in number of flies trapped (females: F=14.0; df = 3, 388; P=0.0001; males: F=6.0; df = 3, 388; P=0.0005). Numbers of females and males trapped by HTY-borax bait were significantly higher than those trapped by any NuLure bait and by NuLure-1% borax bait, respectively (Fig. 3). The pH of the NuLure was directly correlated with the number of flies attracted, as was observed in the laboratory bioassays. Average pH values of the baits in the field trials were 6.1 (10% NuLure-1% borax), 8.2 (10% NuLure-5% borax), 8.4 (10% NuLure-10% borax) and 9.0 (HTY-borax). There was some variation in bait pH at different locations due to the pH of the water used (Table 1). Bait solutions tested at TREC were significantly less alkaline than bait solutions at the other locations (F=30.78; df = 2, 260; P=0.0001). Water pH averaged 7.4 at TREC, but averaged 8.1 at the other locations. Thus, variation in bait pH among the different locations may have affected the outcome of the bait comparisons.

The age of the bait solution did not significantly affect the number of flies trapped. There was, however, a significant interaction between bait and bait age for females (F = 2.71; df = 6, 388; P = 0.01). Therefore, the effect of bait age was examined separately for each of the baits. Bait age significantly affected the catch of females in HTY-borax traps (F = 4.98; df = 2, 100; P = 0.009), but not in NuLure traps. Numbers of both females and males decreased with increasing age of the HTY-borax solution in the field (Fig. 4). This effect was especially pronounced in catches from the Fruit and Spice Park. The average ( $\pm$  SEM) number of flies dropped from 107 ( $\pm$  111) and 72 ( $\pm$  127) in the

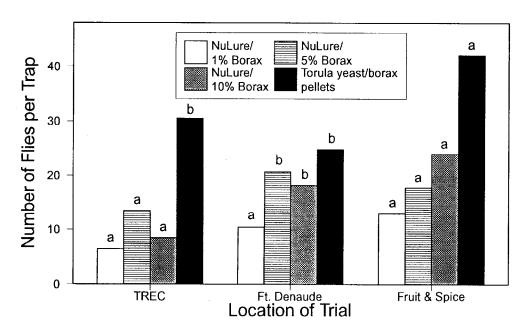


Fig. 2. Average number of female Anastrepha suspensa captured in McPhail traps baited with 10% NuLure with increasing concentrations of borax or with standard HTY-borax pellets (4:5 yeast:borax). Trials were conducted in spring, 1992, at Homestead, FL (TREC [n = 36] and Fruit & Spice [n = 33]) and Ft. Denaude, FL (n = 34). Bars with the same letter within a location are not significantly different (Tukey's mean comparison of log [x + 1] transformed data, P = 0.05).

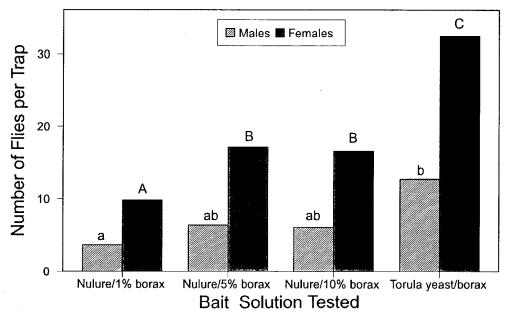


Fig. 3. Average number of male and female Anastrepha suspensa caught in McPhail traps baited with 10% NuLure with increasing concentrations of borax or with standard HTY-borax pellets (4:5 yeast:borax) in spring 1992 field trials in southern Florida (n = 103). Bars with the same letter within a sex are not significantly different (Tukey's mean comparison of  $\log [x + 1]$  transformed data, P = 0.05).

TABLE 1. AVERAGE (STANDARD ERROR) PH VALUE OF BAIT SOLUTIONS USED IN FIELD TESTS AT THREE SITES IN SOUTH FLORIDA (TREC, N=18; Ft. Denaude, N=6; Fr & Sp, N=33).

Aqueous Bait Solution	TREC	Ft. Denaude	Fr & Sp
Water (control)	7.36 (0.23)	8.47 (0.12)	8.12 (0.12)
10% NuLure with 1% borax	5.81 (0.13)	6.45 (0.09)	6.21 (0.08)
10% NuLure with 5% borax	7.78(0.10)	8.00 (0.04)	8.43 (0.05)
10% NuLure with 10% borax	8.12 (0.07)	8.18 (0.03)	8.63 (0.08)
Torula yeast with borax	8.92 (0.04)	8.73 (0.06)	9.08 (0.06)

0-1 d and 2-3 d collections, respectively, to  $5 (\pm 6)$  in the 4-6 d collections. This location had the most trapped flies, and loss in attractiveness may have been due to formation of repellent volatiles from the accumulation of flies in the bait or to loss in attractive volatiles produced by freshly-prepared HTY-borax bait. There was no significant loss in efficacy of HTY-borax solution aged for 6 days prior to field placement in tests of other *Anastrepha* spp. (Malo 1992). Other studies have found that catch increased with age of the HTY-borax bait, but this was attributed to time needed to completely dissolve bait pellets in water (Lopez-D et al. 1968).

Low catch efficiency (Calkins et al. 1984), high cost and difficulties in handling the cumbersome liquid-baited McPhail traps in the field have led to searches for more efficient attractants and traps. However, HTY-borax baited McPhail traps are equal to or can outperform other traps and baits (Mason & Baranowski 1989). This remains true in our tests with NuLure formulations. The two populations with high percentage of unmated female flies were less discriminating, at least among the protein baits offered

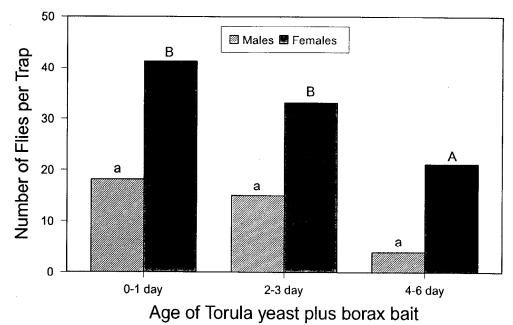


Fig. 4. Effect of bait age on average number of male and female Anastrepha suspensa caught in McPhail traps baited with standard HTY-borax pellets (4:5 yeast:borax) in spring 1992 field trials in southern Florida (n = 36). Fresh bait was made weekly, and recycled after 2 and 4 days. Bars with the same letter within a sex are not significantly different (Tukey's mean comparison of  $\log [x + 1]$  transformed data, P = 0.05).

in this study. In contrast, the population with a high percentage of mated female flies, the population at TREC, made a clear choice between HTY-borax and NuLure. Thus, age structure of the target population may alter response to bait. Bait pH may also be a significant factor that has been overlooked in field tests conducted at different locations or at different times, as changes in water pH as well as bait protein pH may strongly affect trap efficacy. Periodic checks of bait solution pH should be included in trapping protocols. This is very important in trials with acidic protein hydrolysates such as NuLure. Rather than add a predetermined concentration of borax, the amount of borax added should be adjusted to obtain the desired alkaline pH (> 8.0).

Our studies are part of a continuing effort to identify the chemicals responsible for the attraction of caribflies with the goal of developing a synthetic bait that could be used in a dry trap. Preferences of the caribflies in laboratory bioassays correlated with those obtained in field tests. Thus, preferences observed in laboratory bioassays can then be used to indicate the presence of volatile components that warrant further investigation. Identification of attractant volatile chemicals from protein baits is the first phase in developing synthetic lures that optimize cues from these baits to increase catch efficiency in the field.

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# LONGEVITY AND FECUNDITY IN THE CARIBBEAN FRUIT FLY (DIPTERA: TEPHRITIDAE): EFFECTS OF MATING, STRAIN AND BODY SIZE

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#### ABSTRACT

There was no difference in the longevity of mated versus virgin female Caribbean fruit flies, Anastrepha suspensa (Loew), when kept without food. Females provided with food and kept with males lived a shorter period of time than single females, and females with larger males had a shorter lifespan than those kept with smaller males. Females provided with food and caged with large males did not have fecundity greater than those caged with small males. When protein was removed from the female diet, there was no difference between the fecundities of females mated to large and small males. The maximum fecundity of wild females in different size categories was correlated to thorax length. A similar analysis of longevity yielded a relationship that bor-